



## The effect of eugenol and butyric acid glycerides on the qualitative and sensory properties of chicken fillet

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### ABSTRACT

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Due to side effects of growth stimulant antibiotics used for poultry nutrition such as increasing microbial resistance, the poultry industry tries to replace them with a safer one like as phytochemicals and organic acids. Thus, the aim of this research was to investigate the effect of adding butyric acid glycerides (BAG) and eugenol essential oil to the diet of broiler chickens on the quality and sensory properties of the meat. 300 pieces of broiler chickens of commercial strain Ross 308 were bred in the form of a completely random design in six experimental diets including control (T1), T2 (500 ppm eugenol), T3 (1000 ppm eugenol), T4 (BAG 0.2% w/w), T5 (BAG 0.2% w/w + 500 ppm eugenol), and T6 (BAG 0.2% w/w + 1000 ppm eugenol). After 42 days, the broilers were slaughtered and water holding capacity (WHC), drip loss, cooking loss, sensory and pH parameters were evaluated on the breast muscle (fillet) during storage days. The findings revealed that the addition of eugenol and BAG to the diet of broilers reduced drip loss ( $p < 0.05$ ), while indicated no significant effect on the WHC. Incorporation of BAG and eugenol showed insignificant effect on cooking loss, but improved the sensory acceptability of the cooked meat. pH values influenced significantly by eugenol, and ranged between 5.69-5.81. In conclusion, the addition of eugenol and BAG to the diet of broiler chickens improved the sensory properties, and decreased drip loss of the fillets.

## 1. Introduction

Meat can be considered a dense food product that has a wide range of vital nutrients such as proteins, fats and fatty acids. The important point is that all nine essential amino acids needed by humans can be found in meat protein. The distinguishing feature of meat protein from other foods can be considered the presence of valuable amino acids such as histidine, isoleucine, leucine, methionine and tryptophan. Also, the presence of fats and fatty acids such as linoleic acid, linoleic acid and arachidonic acid, which are considered as an energy source for the body, and micronutrients such as phosphates and sulfates show the high value and significant importance of this important product in human life [1]. Among the sources of meat consumption, chicken meat has special importance in human nutrition for certain reasons. Among the reasons that make chicken meat particularly important are the higher protein percentage of chicken meat compared to other meats, less carcass loss after slaughter than beef and sheep meat, limited diseases that can be transmitted from chicken meat to humans, easier digestion. And he pointed out that cholesterol is less and its growth rate is higher. In terms of energy, chicken meat has 165 kcal per 100 grams, compared to mutton which has 180 kcal per 100 grams, it can be recommended for those who are looking for low-calorie diets [2].

Essential oils are volatile oily compounds produced as secondary metabolites by plants. Essential oils have different compositions and effects according to different characteristics such as plant type, growth environment and extraction method. The main reason for paying special attention to essential oils is their antimicrobial, antioxidant, and flavoring properties, which have turned them into suitable and safe alternatives in the food and pharmaceutical industries [3]. The use of essential oils as food flavorings and compounds for the treatment and prevention of infectious diseases has increased in recent years. One of the reasons for this is the trend of consumers towards food products treated with natural and herbal ingredients and the increasing standards of the food industry regarding the type

of additives used in this industry [4] as well as the increasing legal restrictions to ban the use of antibiotics in different countries. [5]. Excessive use of antibiotics causes microbial resistance; For this reason, plant essential oils can be an effective and suitable option for dealing with microbial agents. In the past years, many researches have been done on replacing antibiotics in poultry diet [6]. Recently, essential oils have been in the spotlight for this reason. Essential oils can increase digestive activities, absorb nutrients and improve the nutritional value of the final product. The side effects of using these compounds are very insignificant and this factor has made it possible to use them in various medicinal and food compounds [4]. Eugenol<sup>1</sup> (C<sub>10</sub>H<sub>12</sub>O<sub>2</sub>) is a phenolic compound that can be extracted from a wide variety of plants such as cloves, nutmeg and cinnamon. Having many functional properties has made eugenol a product that has many functional uses. Before this, eugenol is scientifically called from the leaves and buds of the clove plant *Eugenia caryophyllata* they were extracting; But recently, through the allylation reaction of guaicol with allyl chloride, it is produced with similar functional properties [7]. In the field of investigating the effect of using plant essential oils in livestock and poultry diets on growth performance, physiological traits, and qualitative properties of meat, studies have been conducted; In a research by Ghazanfari et al. (2013), they investigated the effects of clove essential oil and antibiotics on growth performance and carcass characteristics of broiler chickens; The results showed that treatments containing clove essential oil caused an increase in carcass and thigh weight [8]. Turco et al. (2020) investigated the effect of grape pomace as a nutritional and antioxidant supplement on unsaturated fatty acids and meat quality; According to the results, the color of breast and thigh was redder in chickens fed with pomace, and the thiobarbituric acid index decreased [9]. In the study of Farias et al. (2020), it was found that the addition of mango seed extract does not have much effect on the antioxidant activity and quality parameters of chicken meat [10]. Hajipour et al. The essential oil of oregano and thyme, separately and in a mixture, decreased the

<sup>1</sup>. Eugenol

amount of thiobarbituric acid, the amount of blood loss and the loss of cooking. Oregano and thyme, due to their high antioxidant properties, increased the antioxidant capacity and ultimately increased the shelf life of meat [11]. Mohib Ali and Moini (2014) investigated the effect of adding different amounts of black mustard seeds to Japanese quail diet. The results showed that increasing the consumption levels of mustard seeds decreased feed consumption in all experimental periods. Dietary treatments had no significant effect on the parameters of thiobarbituric acid, pH, blood loss and water holding capacity [12].

Butyric acid, which is among organic acids, is an important food additive whose beneficial effects on the health of the digestive system of broiler chickens have been proven, and it can be divided into two forms, coated and free [13]. Coated butyric acid is considered a strong and nutritious growth stimulant for poultry, which has various effects such as increasing the level of intestinal absorption, antibacterial activity against pathogenic agents such as *salmonella* And *Escherichia coli* Modulation of intestinal flora, protective effect on beneficial bacteria such as *Lactobacilli* and increasing the digestibility of consumed feed have been mentioned for it [14]. The effect of adding glycerides of butyric acid as a supplement to poultry diet has also been investigated by researchers; Mominizadeh et al. (2019) reported that the addition of butyric acid glycerides supplements to the diet of broiler chickens did not affect the yield of usable carcass, breast, abdominal fat and production index of broiler chickens [15]. In the research of Fakhrabad et al. (2013), it was found that adding glycerides of butyric acid to the diet of chickens has no positive effect on the indicators related to the carcass of broiler chickens [13]. Bedford et al. (2017) observed that by adding butyrate glycerides to the diet of broiler chickens, a stable change in fat metabolism occurred, and the combination of supplements with moderate levels caused an increase in breast weight [16].

Based on the available sources, the research conducted on phytochemical compounds in poultry nutrition has been increasing in the last few years.

On the other hand, the investigation of the synergistic effects of these compounds with other additives, including organic acids, on production, physiological and meat quality traits has been done in a very limited way. Therefore, there is room for more research in this field. Therefore, in this research, an effort is made to investigate the effects of adding eugenol essential oil and butyric acid glycerides to the diet of broiler chickens on some qualitative and sensory characteristics of breast muscle meat.

## 2- Materials and methods

### 2-1-glycerides of butyric acid and eugenol

Glycerides of butyric acid with the brand name C4-BaBy (monoglyceride of butyric acid (25-35%), diglyceride of butyric acid (50-55%), triglyceride of butyric acid (15-25%)) from Sena Dam Pars, clove oil (contains 86 % eugenol) was obtained from Abatage Pharmaceutical and Trading Company (Ait Essence).

### 2-2- Breeding and preparation of broiler chickens

In this research, 300 pieces of one-day-old broilers of Ras strain<sup>2</sup> 308 with similar average weight were randomly assigned to six food treatments in five replicates and ten bird pieces per replicate. At the end of the 42-day breeding period, one bird for each replication with a weight close to the average weight of the corresponding replication was selected and killed. In animal experiments, the environmental conditions (temperature, humidity, light intensity, etc.) were controlled according to the recommendations of the strain management guide. The light program was applied in the form of 24-hour lighting at the beginning of the period and then 16 hours of light and 8 hours of darkness, and vaccination was carried out according to the program recommended by the veterinary organization of Ardabil province. Access to water and soil was free from one day until the end of the period, except during the recording times (Table 1). The experimental diets contained two levels of butyric acid glycerides (zero and 0.2% w/w) and three levels of eugenol (zero, 500 and 1000 ppm) and

<sup>2</sup>1. Ross

were as follows:

- 1- Witness ration
- 2- Diet containing 500 ppm eugenol
- 3- Diet containing 1000 ppm eugenol
- 4- Diet containing 0.2% glycerides of butyric acid
- 5- Diet containing 0.2% glycerides of butyric acid and 500 ppm eugenol
- 6- Diet containing 0.2% glycerides of butyric acid and 1000 ppm eugenol

To apply experimental treatments, the amount of eugenol required according to the amount of ration was weighed in grams and mixed with ration oil and then completely combined with the rest of the ration. In the case of butyric acid glycerides, the required amount in grams was first

mixed with corn in the diet and then added to the rest of the diet.

After 42 days of rearing, the chickens were slaughtered in the slaughter department of the rearing hall by cutting the neck and skinning and emptying the intestines and viscera on each carcass. Each carcass was divided into two pieces and the breast meat (fillet) was selected and separated for experiments. The fillets were washed with city water in the poultry house and after complete dewatering and drying, they were packed in zip packs and placed in ice and placed in the laboratory refrigerator with a transfer temperature of 4°C.

**Table 1** The ingredients and chemical composition of experimental diets during different periods.

Ingredients (%)	Starter	Grower	Finisher
Maize	52.83	56.40	60.28
Soybean meal	38.98	35.50	31.63
Vegetable oil	3.46	3.88	3.98
Oyster shell	1.27	1.06	1.07
Dicalcium phosphate	1.87	1.69	1.68
Common salt	0.41	0.41	0.38
Vitamin premix <sup>1</sup>	0.25	0.25	0.25
Mineral premix <sup>2</sup>	0.25	0.25	0.25
DL-Methionine	0.36	0.27	0.25
L-Lysine hydrochloride	0.28	0.25	0.19
Total	100	100	100
MEn (Kcal/kg)	2975	3050	3100
Crude protein (%)	22.12	20.81	19.37
Lysine (%)	1.41	1.30	1.16
Methionine (%)	0.69	0.59	0.55
Methionine + Cysteine (%)	1.05	0.93	0.87
Arginine (%)	1.42	0.32	1.22
Calcium (%)	1.04	0.91	0.90
Available phosphorous	0.49	0.45	0.44
Sodium (%)	0.18	0.18	0.17

1. Provided per kg of diet: Vit A: 9000 IU, Vit D3: 2000 IU, Vit E: 36 mg, Vit K3: 2 mg, Vit B1: 1.75 mg, Vit B2: 6.6 mg, Calcium pantothenate: 9.8 mg, Niacin: 10 mg, Vit B6: 2.94 mg, Vit B9: 1 mg, Vit B12: 0.015 mg, Choline chloride: 250 mg, Antioxidant: 1 mg.

2. Provided per kg of diet: Mn: 99.2 mg, Zn: 84.7 mg, Fe: 50 mg, Cu: 10 mg, I<sub>2</sub>Se : 0.99 mg, Se : 0.2 mg.

## 2-3- pH

pH was measured by a pH probe meter (Testo 205 model, Germany) at a certain place on the fillet at two points at a depth of 1.5 cm at 24 hours after slaughter. Before starting the measurement, the meat samples were taken out of the refrigerator to reach the ambient temperature [17].

## 2-4-Water holding capacity (WHC)

To determine WHC, the blood level measurement

method (output humidity) was used; Moisture output on 2nd, 5th and 7th days after Barushpujavinivara slaughter<sup>3</sup> (1957) was measured. The amount of 0.3 grams of minced meat was weighed by an electric grinder (model 491 FU FUMA, Japan) and placed on Whatman filter paper.<sup>4</sup> Number one (9 cm) was placed. Then, filter paper was transferred to a plastic filter and another filter was placed on the meat sample. A two-kilogram weight was placed exactly on the

<sup>3</sup>1Pohia&Niinivaara

<sup>4</sup>. Whatman

sample for 2 minutes in the center of Talaq. In the method of determining the leachate area, after the weighing time, the filter paper with meat paste was scanned and saved by a scanner (Canon LiDE 120 model, Japan) with 300 dpi quality. Then, the area of the leachate of the meat area and its surroundings was calculated by Digimizer software (version 5.4.9) and put in the following relationship to obtain WHC. In this regard, A is the area of the blood vessel under the pressed meat (cm<sup>2</sup>) and B the total area of the blood vessel (cm<sup>2</sup>) Is:

$$WHC = B - A/A$$

## 5-2- Blood loss

Hanikel's (1998) method was used to determine blood loss in meat on days 2, 4, and 6 after slaughter. About 40 grams of meat with a thickness of 2 to 2.5 cm was placed in a plastic net and the net containing the meat was hung inside a cylindrical container with a lid; The lid of the container was closed and kept in the refrigerator to measure at the mentioned times. Blood loss was calculated with the following equation:  $W_1$  Meat weight on the day before measurement and  $W_2$  The weight of the meat on the day of measurement (days 2, 4 or 6 of storage in the refrigerator) is:

$$= \text{blood loss } W_1 - IN_2 / IN_1 \times 100$$

## 2-6- cooking drop

The cooking loss was determined on the third day after slaughter by the method of Hanikel (1998). For this purpose, about 50 grams of meat sample with a thickness of 1.5 cm was weighed and transferred into a zip-pack plastic bag. Then, the bags were placed in a bain-marie with a temperature of 80°C for one hour to cook the meats. After this time, the bags were removed from the bain-marie and cooled under running water. Take the meat out of the package and dry it with a towel. The cooking value was determined with the following relation, that  $W_1$  The initial weight of meat and  $W_2$  The weight of the meat after cooking is:

$$= \text{cooking loss } W_1 - IN_2 / IN_1 \times 100$$

## 2-7- Blood loss

Hanikel's method (1998) was used to determine blood loss in meat on days 2, 4 and 6 after

slaughter. About 40 grams of meat with a thickness of 2 to 2.5 cm was placed in a plastic net and the net containing the meat was hung inside a cylindrical container with a lid. The lid of the container was closed and kept in the refrigerator to measure at the mentioned times. Blood loss was calculated with the following relationship that  $W_1$  Meat weight on the day before measurement and  $W_2$  The weight of the meat on the day of measurement (days 2, 4 or 6 of storage in the refrigerator) is:

$$= \text{blood loss } W_1 - IN_2 / IN_1 \times 100$$

## 8-2- Sensory test

A five-point hedonic test was used to check the sensory characteristics of meat. For this test, 10 trained referees from the students of Mohaghegh Ardabili University, who were in the age range of 20-30 years, were used. Each subject was given a three-digit code randomly. Then, the judges were asked to consume the meats that were the result of the cooking loss test and assign a score of 1 (very bad) to 5 (very good) in the respective forms for each of the characteristics of color, smell, taste, mouthfeel and overall acceptance. In the interval between taking each treatment, the judges drank water to remove the traces of the previous sample from the mouth.

## 2-9 statistical analysis

The experiments were conducted in a completely randomized design. The results of measurements using one-way analysis of variance tests<sup>5</sup> Independent and one-way ANOVA with repeated measures<sup>6</sup> (repetition in time) for the tests that were repeated over time, were analyzed using SAS software (9/4 version). Comparison of means was done by Duncan's test and at a probability level of less than 5%.

## 3. Results and Discussion

### 3-1-pH

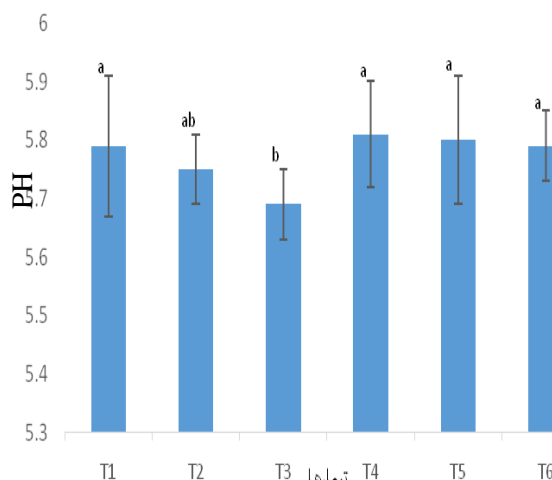
The pH value of meat indicates acidity and its effects on color and water loss. Meat pH has been considered as an indicator related to glycogen and its changes after slaughter are considered as factors of glycogen decomposition and lactate production[18]. In this study, the pH of meat samples was measured 24 hours after the initial

<sup>5</sup>. One way-ANOVA

<sup>6</sup>. Repeated measurement



slaughter and the pH ranged from 5.69 to 5.81. The results of the analysis of variance of the data indicated a significant difference in the pH level ( $p < 0.05$ ). According to the results in the diets with eugenol, the pH of the samples decreased so that the pH value of the sample with 1000ppm of eugenol was the lowest, but with the addition of glyceride of butyric acid to the diet, The pH value of the treatments increased again and the presence of eugenol (treatments T5 and T6) did not affect it either (Figure 1). Miller and Warrell (2004) compared the effect of adding thymol and eugenol to cattle diets. They observed that in treatments with eugenol, the pH of the meat decreased faster and the final pH value was lower. In justifying this phenomenon, they stated that eugenol creates an effect similar to the accumulation of lactate in the tissue and will cause the pH to decrease [19]. Considering that eugenol is a weak acid (eugenic acid), it is likely that those eugenol molecules that reach the cells cause a decrease in pH. In another study, Popovich et al. Breasts were tested 24 hours after slaughter. In the treatments that used a mixture of essential oils in the diet, the pH was higher than the control treatment, although this difference was not significant. They stated that the pH range of 5.92 to 6.2 in breast muscle is a standard range in terms of creating the appropriate color and texture [20]. Agus et al. (2019) investigated the effect of adding a mixture of nine essential oils to the drinking water of broilers. The range of pH was reported between 6.18 and 6.20, and the group with the additive had a slightly higher pH [21]. Simon et al. (2014) measured the effect of adding cinnamon to the diet of Cobb 700 broiler chickens and measured the pH at 24 hours after slaughter. Their pH range is between 5.82-5.76 and the treatment with a higher dose of cinnamon was able to obtain the highest pH value [22]. Ipkach et al. (2018) studied the effect of a mixture of compounds that can be found in essential oils on a number of Ras 308 strain broilers.



**Fig 1** Fillet pH of broiler chickens fed by diets containing different levels of eugenol and butyric acid glycerides at 24h postmortem (T1: control, T2: 500 ppm eugenol, T3: 1000 ppm eugenol, T4: 0.2% w/w butyric acid glycerides, T5: 0.2% w/w butyric acid glycerides + 500 ppm eugenol, T6: 0.2% w/w butyric acid glycerides + 1000 ppm eugenol). Different lowercases and uppercases on the top of bars indicate significant differences ( $p < 0.05$ ) within a day of storage (among treatments) and during storage, respectively.

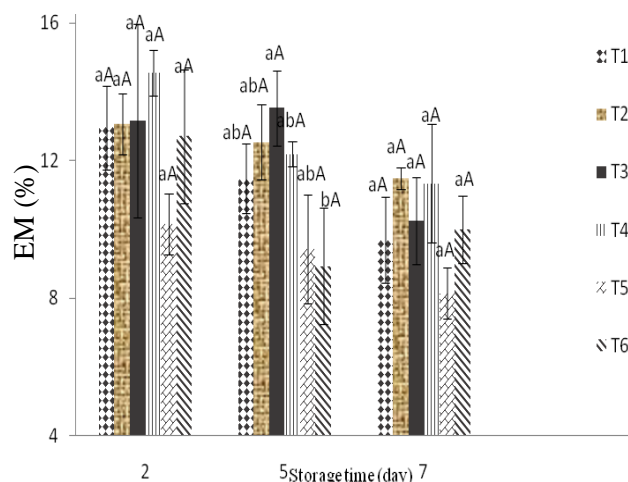
Although the pH was measured 15 minutes after slaughter, the difference was not significant and the pH value of the meat samples was between 5.69 and 5.76 [23]. In the present study, the presence of glycerides of butyric acid in the diet did not lead to a significant difference in the pH value of meat. Similarly, Gomati et al. (2018) tested the effect of sodium butyrate plus cinnamon oil on the diet of broiler chickens and found no significant difference for pH at 24 h post-mortem. Besides, their pH range was between 5.98-5.72 [24].

### 3- 2- Water holding capacity (WHC)

The ability to retain water by meat or water added to it against external pressures is called water holding capacity or WHC [25]. The water holding capacity has an inverse relationship with another index called outlet moisture (leachate) or EM. Water holding capacity affects the retention of minerals, vitamins and the volume of water released by the tissue [26]. The results of the analysis of variance showed that the passage of time did not have a significant effect on the moisture content of the meats ( $p < 0.05$ ), although

the overall value of this parameter showed a decreasing trend during the measurement days (Figure 2). In fact, with the passage of time and the ripening of the meat, as a result of the breakdown of proteins and also the increase in load due to the absorption of potassium ions and the release of calcium ions, the osmotic pressure increases and leads to an increase in WHC (reduction of output moisture) [27]. The amount of drop is decreasing with the passage of time.

The results of the analysis of variance of the data indicated that there was no significant difference between the output moisture of the samples on the second and seventh days ( $p < 0.05$ ), but on the fifth day, the type of feed additive created a significant difference in the output moisture and the meat of the chickens fed with the diet containing 0.2% butyric acid glyceride and 1000 ppm eugenol had the lowest amount of output moisture ( $p < 0.05$ ). In general, the combined use of two additives showed better results and the output moisture content was lower than the control treatment, while the use of only one of the additives had worse results than the control treatment, and the output moisture content was numerically higher than the control in all days. Although in the present study, eugenol essential oil when used with butyric acid glycerides had a positive effect on increasing WHC, but the researchers' findings show that essential oil alone is able to reduce the amount of moisture coming out of meat. For example, Popovich et al. (2019) in a study measured the effect of a mixture of edible essential oils on the juice retention capacity of the breast meat of Ross strain broilers. The results showed that the treatments in which essential oils were used had higher WHC than the control treatment [20]. In another research, Agus et al. (2019) added a mixture of 9 essential oils to the feed water of broiler chickens and observed that treatments with essential oils had significantly higher WHC than the control treatment [21].



**Fig 2** Expressed moisture (EM) values of broiler chickens fed by diets containing different levels of eugenol and butyric acid glycerides at different times of storage (T1: control, T2: 500 ppm eugenol, T3: 1000 ppm eugenol, T4: 0.2% w/w butyric acid glycerides, T5: 0.2% w/w butyric acid glycerides + 500 ppm eugenol, T6: 0.2% w/w butyric acid glycerides + 1000 ppm eugenol). Lowercases and uppercases on the top of bars indicate significant differences ( $p < 0.05$ ) within a day of storage (among treatments) and during storage, respectively.

Waris et al. (2000) found that essential oils (thyme and oregano) prevent meat oxidation due to their antioxidant properties and thus increase the WHC of chicken meat [28]. The results of measuring the WHC of meats by measuring the area of moisture (leach) output on different days are presented in Table 2. Also, it is clear that the type of additive to the ration and days of maintenance did not have a significant effect on WHC ( $p < 0.05$ ). The smaller this number is, the less moisture has been removed from the meat. Similar to the output moisture, in all treatments, with the passage of time and due to the applied pressure, less amount of leachate was removed, which is a sign of an increase in WHC. The results of the Pearson correlation test showed that there is a positive and significant correlation between the data obtained from the measurement of the output moisture weight (EM) and the determination of the output moisture area (leachate). In other words, by reducing the amount of EM, a corresponding surface reduction occurs on the filter paper, both of which can indicate changes in the WHC of the meat.

Therefore, both weight or surface determination methods can be used to determine WHC. In contrast to EM, the correlation between changes

in water table drop and outlet leachate area was insignificant (Table 3).

**Table 2** Measurement results of expressed moisture area exudate from fillet of broiler chickens fed by diets containing different levels of eugenol and butyric acid glycerides at different times of storage \*.

7	5	2	Day Treatment**
1.14±0.71 <sup>aA</sup>	1.16±0.91 <sup>aA</sup>	2.02±0.35 <sup>aA</sup>	T1
1.18±0.69 <sup>aA</sup>	1.93±0.92 <sup>aA</sup>	2.15±0.26 <sup>aA</sup>	T2
1.42±0.40 <sup>aA</sup>	2.38±0.68 <sup>aA</sup>	2.67±0.88 <sup>aA</sup>	T3
1.67±0.41 <sup>aA</sup>	1.91±0.23 <sup>aA</sup>	2.20±0.25 <sup>aA</sup>	T4
0.90±0.25 <sup>aA</sup>	1.33±0.42 <sup>aA</sup>	1.79±0.24 <sup>aA</sup>	T5
1.56±0.35 <sup>aA</sup>	1.37±0.16 <sup>aA</sup>	2.19±1.06 <sup>aA</sup>	T6

\*: Means with the same lowercases within a column indicate insignificant differences on a day, and means with the same uppercases within a row indicate insignificant differences during storage.

\*\* : T1: control, T2: 500 ppm eugenol, T3: 1000 ppm eugenol, T4: 0.2% w/w butyric acid glycerides, T5: 0.2% w/w butyric acid glycerides + 500 ppm eugenol, T6: 0.2% w/w butyric acid glycerides + 1000 ppm eugenol.

**Table 3** Pearson's correlation coefficient of the parameters linked with water holding capacity.

Drip loss	Expressed moisture	Parameters
0.25	0.43**	Area of expressed moisture
-	0.28*	Drip loss

\*: Significant at  $p < 0.95$

\*\* : Significant at  $p < 0.99$

### 3-4- Blood loss

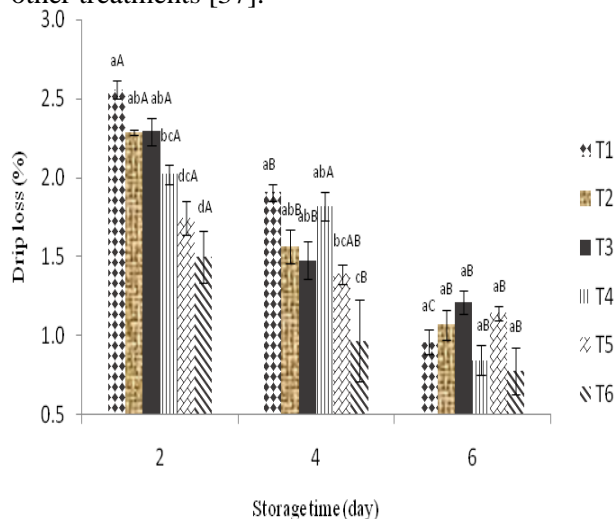
Bleeding can be considered as a liquid that is secreted from it without any pressure or mechanical force and only due to the weight of the meat, and water and protein can be considered as its two major components [29]. Blood loss causes a decrease in the economic value of meat due to weight loss (2-10%) and its inappropriate shape due to the presence of exudates around the meat [30]. The speed and amount of blood formation in meat is influenced by factors such as dead body, wrinkling, permeability to water and abnormality [31]. In the analysis of blood loss data, a significant difference was obtained between the treatments ( $p < 0.05$ ). On the 2nd and 4th days of measurement, the control treatment (T1) had the highest amount of blood seepage (Figure 3). The amount of drop in the samples containing only eugenol or only glycerides of butyric acid did not show a significant difference. When these two substances were used in feeding broilers, the amount of blood loss continued to decrease and in the diet containing the highest amount of eugenol and glyceride of butyric acid (T6) it was significantly lower than the control sample, which decreased by 35% on the second

day. According to Hoff et al.'s (2005) theory, the characteristics related to tissue water, such as WHC and blood loss, can be related to proteolysis, and since essential oils such as thyme, cloves, and oregano have antioxidant properties, by maintaining the proteolytic activity of enzymes and increasing tissue alkalinity, they cause water retention. They are woven [32]. The passage of time had a significant effect on the amount of blood loss in the meats ( $p < 0.05$ ) and until the last day of measurement, the blood was still coming out of the meat, but in terms of weight, it was less than the first days. The amount of decline over time was also less compared to the control and the effectiveness order of the treatments did not change much during the test period. Among the reasons for the decrease in blood pressure with the passage of time can be related to the slight increase in pH, as a result of this increase, the myofibrillar proteins move away from the isoelectric pH and the electrostatic repulsion between the filaments increases, which causes the filaments to move away from each other, increasing the space between the filaments and Reducing the amount of water is released [17]. The positive effect of edible essential oils



and organic acids on reducing the amount of blood loss in meat has been proven in some other researches. For example, Sugiharto et al. (2019) in a research titled the effect of formic acid and butyric acid on the characteristics of broiler chicken carcasses found that butyric acid is able to reduce blood loss [33]. In the research of Hajipour et al. (2014), the effect of thyme and oregano on blood loss in broiler chickens was studied, and the amount of reduction in blood loss under the effect of thyme and oregano essence was reported to be significant compared to the control treatment and the antibiotic treatment [11]. In the research of Shirzadi et al. (2019), the use of Sarkhar Gol caused a slight decrease in the amount of blood loss in the thigh muscle of broiler chickens [34]. Sheikhsamani et al. (1400) compared the effect of oregano and rosemary in the diet of quail chicks and observed that blood loss due to oregano was significantly lower than that of rosemary and the control treatment [35].

The use of various additives in the food ration does not always have a significant effect on blood loss; in an experiment conducted by Ray et al. (2017), oregano essential oil added to the food ration did not affect the blood loss of meat [36]. In the research conducted by Rajabi et al. (2019) by adding rosemary and oregano powder and essential oil to the diet of broiler quail chicks, they did not observe a significant difference in blood loss between the control treatment and other treatments [37].



**Fig 3** Drip loss values of fillet of broiler chickens fed by diets containing different levels of eugenol and butyric acid glycerides at different times of storage (T1: control, T2: 500 ppm eugenol, T3: 1000 ppm

eugenol, T4: 0.2% w/w butyric acid glycerides, T5: 0.2% w/w butyric acid glycerides + 500 ppm eugenol, T6: 0.2% w/w butyric acid glycerides + 1000 ppm eugenol). Different lowercases and uppercases on the top of bars indicate significant differences ( $p < 0.05$ ) within a day of storage (among treatments) and during storage, respectively.

### 3-5- cooking loss

Cooking loss can be considered as a combination of solid and liquid dissolved substances that are removed from the meat during the cooking process [38]. High cooking loss is one of the factors affecting the quality properties of meat such as juiciness, mouth feel and sensory acceptance. The results of cooking loss are presented in Table 3, and it shows that the difference in data is not significant ( $p < 0.05$ ). The data showed that the average cooking loss of the treatments had near numerical values. Similar to this research, some researches have also reported the ineffectiveness of supplementing the diet with additives and especially essential oils on the loss of meat cooking; Gomati et al. (2018) evaluated the effect of cinnamon essential oil and sodium butyrate in quail broilers and found no significant difference between the treatments and the control treatment [24]. Shirzadiouhmakaran (2019) investigated the effect of hyssop thyme powder essential oil on the cooking loss of the thigh muscle of broiler chickens and did not observe any significant effect [34]. In a study, Hajipour et al. (2014) tested the effect of thyme and oregano essential oil on the cooking loss of quail chicken meat. They attributed the lowest amount of cooking loss to the thyme treatment and stated the reason for this is the antioxidant property of thyme, which improves WHC and reduces cooking loss [11]. In a number of studies, the cooking loss parameter has been significantly affected by the changes in the animal diet; In the research of Popovich et al. (2019), which was conducted using a mixture of essential oils, the amount of cooking loss decreased. They considered the reason for the drop to be the decrease in the amount of WHC in meat due to the denature of proteins at low pH values [20]. Agus et al. (2019) investigated the addition of oregano and thymol essential oils to the drinking water of broiler chickens and observed that the cooking loss of the samples increased [21]. In

another study, the use of a mixture of essential oils in the food ration reduced cooking loss[23].

**Table 3** Cooking loss values (%) obtained for fillet of broiler chickens fed by diets containing different levels of eugenol and butyric acid glycerides on day 3.

T6	T5	T4	T3	T2	T1	Treatment*
28.88±2.83 <sup>a</sup>	28.81±3.22 <sup>a</sup>	28.24±1.54 <sup>a</sup>	29.68±4.29 <sup>a</sup>	29.69±1.02 <sup>a</sup>	29.80±1.28 <sup>a</sup>	Cooking loss (%)

\*: T1: control, T2: 500 ppm eugenol, T3: 1000 ppm eugenol, T4: 0.2% w/w butyric acid glycerides, T5: 0.2% w/w butyric acid glycerides + 500 ppm eugenol, T6: 0.2% w/w butyric acid glycerides + 1000 ppm eugenol.

### 6-3- Sensory tests

Sensory evaluation can be considered as a step that has a significant role in the food industry. Understanding the relationship between the characteristics of food and its acceptance by consumers and acceptance by buyers is one of the undeniable indicators of food industry knowledge. In the sensory test, five characteristics of color, smell, taste, mouthfeel and acceptance were evaluated by the judges. The analysis of the results showed that there is no significant difference in the characteristics of color, mouthfeel and overall acceptance between different meat treatments ( $p < 0.05$ ) and only the characteristics of smell and taste showed a significant difference ( $p < 0.05$ ). The results of the odor evaluation showed that the diet containing butyric acid glycerides and 500 ppm eugenol containing only butyric acid glycerides were more acceptable among the judges. Regarding the taste, the situation was similar and the two rations received higher scores. On the whole, chicken meat that was fed only glycerides of butyric acid, as well as the combination of eugenol and glycerides of butyric acid, had more sensory acceptability among the judges in all five evaluated characteristics than other meats, and the addition of glycerides of butyric acid resulted in more points. was (Table 4). But unlike glycerides of butyric acid, the presence of eugenol decreased the scores of the judges in most of the sensory properties, although this decrease was meaningless. There are 5 types of fat in the animal body, intercellular fat (marbling fat) is effective on the taste and crispiness of the meat. Probably, the presence of butyric acid glycerides through slight changes in the

composition of this type of fat has improved the sensory characteristics of meat [39].

Samadian et al. (2012) reported that adding thyme, lemon and mint essential oils improves the taste and smell of meats. They cited the oxidation of meat fat and the decomposition of a wide range of substances such as aldehydes, ketones, and hydrocarbons as the cause of the smell and taste, and considered the antioxidant properties of oil essences effective on the taste and smell and better acceptance of cooked broiler chickens [40]. Popovich et al. (2019) investigated the effect of adding essential oils of rosemary, oregano and thymol on the sensory properties of broiler chicken breast and observed that the mixture of essential oils obtained significantly higher scores and concluded that the addition of oil essential oils to the diet of broiler chickens improved The quality of the meat will be produced [20]. The sensory test results of Ipkach et al. (2018) on chickens fed with a diet containing essential oils indicated an improvement in the crispness and wateriness of the thigh muscle and no significant change in the sensory quality of the breast muscle. They considered the cause of this phenomenon to be the combination of different essential oils and their synergistic effect [23]. In the research of Casares et al. (2018), oregano essential oil increased the score of sensory properties of broiler meat, but the effect was not significant. They considered the reason for this higher score to be the antioxidant properties and having polyphenol and flavonoid essential oils, which limit the oxidation of lipids and proteins [41]. In another study, the addition of essential oils of Mexican yipone oil to the drinking water of broiler chickens improved the properties of smell, taste and overall acceptance of the meat [42].

**Table 4** Results of sensory evaluation for cooked fillet of broiler chickens fed by diets containing different levels of eugenol and butyric acid glycerides\*.

T6	T5	T4	T3	T2	T1	Treatment**	Day
4.28±0.76 <sup>a</sup>	4.28±0.95 <sup>a</sup>	4.42±0.79 <sup>a</sup>	4.14±0.38 <sup>a</sup>	4.14±0.38 <sup>a</sup>	3.85±0.38 <sup>a</sup>	Color	
3.85±0.90 <sup>ab</sup>	4.42±0.53 <sup>b</sup>	4.00±0.58 <sup>ab</sup>	3.85±1.07 <sup>ab</sup>	3.00±1.00 <sup>a</sup>	3.57±0.79 <sup>a</sup>	Odor	
4.00±0.82 <sup>ab</sup>	4.14±0.69 <sup>b</sup>	4.57±0.53 <sup>a</sup>	3.71±1.25 <sup>ab</sup>	3.14±0.69 <sup>a</sup>	3.85±0.69 <sup>ab</sup>	Taste	
4.14±0.69 <sup>a</sup>	3.85±0.69 <sup>a</sup>	4.14±0.90 <sup>a</sup>	3.57±0.79 <sup>a</sup>	3.42±0.79 <sup>a</sup>	3.71±0.76 <sup>a</sup>	Oral acceptance	
4.00±0.82 <sup>a</sup>	4.28±0.49 <sup>a</sup>	4.28±0.76 <sup>a</sup>	3.71±1.11 <sup>a</sup>	3.57±0.53 <sup>a</sup>	3.85±0.47 <sup>a</sup>	Overall acceptability	

\*: Means with different lowercases within a row indicate significant differences ( $p < 0.05$ ).

\*\* : T1: control, T2: 500 ppm eugenol, T3: 1000 ppm eugenol, T4: 0.2% w/w butyric acid glycerides, T5: 0.2% w/w butyric acid glycerides + 500 ppm eugenol, T6: 0.2% w/w butyric acid glycerides + 1000 ppm eugenol.

## 4 - Conclusion

The results showed the parameters WHC and cooking loss. Under the influence of the addition of eugenol and glycerides of butyric acid was not included in the diet of broiler chickens, but the amount of blood loss was positively affected and reduced. The pH value also showed a decrease only in diets with eugenol, which can be important in terms of increasing the storage time. The sensory evaluation test proved that only the smell and taste of the meats were positively affected. By examining the effect of these substances on other qualitative, physical and chemical characteristics of the breast and thigh muscles, a better conclusion can be reached whether to improve the meat characteristics, the diet of chickens with Should eugenol and butyric acid glycerides be modified or not?

## 5-Resources

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مقاله علمی-پژوهشی

## تأثیر اوژنول و گلیسیریدهای اسید بوتیریک بر خواص کیفی و حسی گوشت جوجه‌های گوشتی

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## اطلاعات مقاله

## چکیده

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به دلیل عوارض مصرف آنتی‌بیوتیک‌های محرک رشد در تغذیه طیور از قبیل افزایش مقاومت میکروبی، صنعت طیور به دنبال جایگزین کردن این مواد با ترکیباتی مانند فیتوجنیک‌ها و اسیدهای آلی می‌باشد. لذا هدف از این تحقیق، بررسی اثر افزودن گلیسیریدهای اسید بوتیریک و اسانس اوژنول به جیره غذایی جوجه‌های گوشتی بر خواص کیفی و حسی گوشت بود. تعداد ۳۰۰ قطعه جوجه گوشتی سویه تجاری راس ۳۰۸ در قالب طرح کاملاً تصادفی و در شش جیره آزمایشی شامل جیره شاهد (T1)، (حاوی ۵۰۰ ppm اوژنول)، T3 (حاوی ۱۰۰۰ ppm اوژنول)، T4 (حاوی ۰/۲٪ w/w گلیسیریدهای اسید بوتیریک)، T5 (حاوی ۰/۲٪ w/w گلیسیریدهای اسید بوتیریک و ۵۰۰ ppm اوژنول) و T6 (حاوی ۰/۲٪ w/w گلیسیریدهای اسید بوتیریک و ۱۰۰۰ ppm اوژنول) پرورش یافت. پس از گذشت ۴۲ روز، جوجه‌های گوشتی کشتار و آزمون‌های pH، ظرفیت نگهداری آب (WHC)، افت خونابه، افت پخت و ارزیابی حسیدر روزهای معین رویعضله سینه (فیله) انجام شد. یافته‌ها نشان داد که افزودن اوژنول و گلیسیریدهای اسید بوتیریک به جیره غذایی جوجه‌های گوشتی موجب کاهش افت خونابه در فیله‌ها شد ولی تأثیر معنی‌داری بر WHC ایجاد نکرد. افزودن گلیسیریدهای اسید بوتیریک و اوژنول روی افت پخت بی‌تأثیر بوده ولی مقبولیت حسی گوشت پخته را بهبود داد. pH فیله‌های مرغ بصورت معنی‌داری تحت تأثیر قرار گرفته و در دامنه ۵/۶۹-۵/۸۱ قرار گرفت. در مجموع، افزودن اوژنول و گلیسیریدهای اسید بوتیریک به جیره غذایی جوجه‌های گوشتی منجر به کاهش افت خونابه و بهبود نسبی ویژگی‌های حسی گوشت شد.

کلمات کلیدی:

اسانس روغنی،

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